

REPORT DOCUMENTATION PAGE					Form Approved OMB No. 0704-0188	
<p>The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing the burden, to the Department of Defense, Executive Service Directorate (0704-0188). Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.</p> <p>PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ORGANIZATION.</p>						
1. REPORT DATE (DD-MM-YYYY) 01-08-2012		2. REPORT TYPE Final Report			3. DATES COVERED (From - To) Sept 30, 2010 - Mar 29, 2012	
4. TITLE AND SUBTITLE Toxic Effects of Lead and Electromagnetic Pulse Energy on Memory Mechanisms					5a. CONTRACT NUMBER	
					5b. GRANT NUMBER FA9550-10-1-0547	
					5c. PROGRAM ELEMENT NUMBER	
					5d. PROJECT NUMBER	
6. AUTHOR(S) Douglas S. F. Ling, Ph.D. Lie Yang, Ph.D. Ronald G. Riechers, Ph.D. Edward Moshang, MSEE					5e. TASK NUMBER	
					5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) SUNY Downstate Medical Center, 450 Clarkson Ave., Brooklyn NY 11203 EMTech Consultants Inc., 1222 Meadowlark Drive, Baltimore, MD 21286 Spectral Energetics, 1306 Research Drive, Beaver Creek, OH 45432					8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Air Force Office of Scientific Research, 875 North Randolph Street, Arlington, VA 22203 Defense Advanced Research Projects Agency, 3701 North Fairfax Drive, Arlington, VA 22203 A					10. SPONSOR/MONITOR'S ACRONYM(S) AFOSR; DARPA	
					11. SPONSOR/MONITOR'S REPORT NUMBER(S) AFRL-OSR-VA-TR-2012-0933	
12. DISTRIBUTION/AVAILABILITY STATEMENT Available for public release; distribution unlimited.						
13. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the authors and should not be construed as an official position, policy, or decision of the Department of Defense, the Department of the Air Force, or DARPA, unless so designated by other documentation.						
14. ABSTRACT Traumatic brain injury (TBI) due to explosive blast is a signature wound of combat operations, but the etiology of blast TBI is still not fully elucidated. The goal of this study was to determine whether low-level electromagnetic pulse (LEMP) energy, alone or in combination with lead (Pb), disrupts the physiological mechanisms of memory, and thus represents a potential neuropathogenic factor of explosive blasts. We examined the effects of LEMP and Pb on long-term potentiation (LTP), which is thought to be a key process of memory, using acutely prepared slices of rat hippocampus. A mathematical model of blast-generated LEMP was developed to estimate the EM energy received by the brain from a 10 kg blast from a 10 m distance and then scale the values for brain slices. An EM pulse generation system was used to expose slices to these LEMP values. In the absence of Pb, we found that single, brief (1 ms) exposures to 800 MHz or 1000 MHz LEMP significantly inhibited LTP induction, whereas 650 MHz LEMP had no effect. However, in the presence of low-level Pb (10 uM), 650 MHz LEMP effectively blocked LTP. These results suggest that LEMP may represent a pathogenic factor of blast TBI, and that low levels of Pb may increase neural susceptibility to LEMP.						
15. SUBJECT TERMS electromagnetic pulse, Pb, lead acetate, long-term potentiation, synaptic transmission, electrophysiology, hippocampus, rat, brain slice						
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON Douglas S. F. Ling, Ph.D.	
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified			19b. TELEPHONE NUMBER (Include area code) 718-270-1349	

Award Number: FA9550-10-1-0547

TITLE: “ Toxic Effects of Lead and Electromagnetic Pulse Energy on Memory Mechanisms”

INVESTIGATORS:

Principal Investigator:

Douglas S. F. Ling, Ph.D.

Associate Professor of Physiology and Pharmacology

SUNY Downstate Medical Center

Investigator: Lie Yang, M.D., Ph.D., Research Assistant Professor,
SUNY Downstate Medical Center

Collaborating Investigators:

Edward Moshang, MSEE, EMTech Consultants, Inc.

Ronald G. Riechers, Ph.D., Spectral Energetics

REPORT DATE: 08/01/12

TYPE OF REPORT: Final

PREPARED FOR: The Air Force Office of Scientific Research and the Defense Advanced
Research Projects Agency

The views, opinions, and/or findings contained in this report are those of the authors and should not be construed as an official position, policy, or decision by the Department of Air Force, Defense Advanced Research Projects Agency, Department of Defense, or the U.S. Government, unless so designated by other documentation.

Table of Contents

Cover.....	1
Introduction.....	3
Specific Aims and Experimental Approach.....	4
Changes to Originally Proposed Aims.....	4
Experimental Methods and Results.....	5
Task 1: Mathematical Model of Explosive Blast-generated LEMP.....	5
Task 2: Design and Testing of Laboratory EM Pulse Generation System.....	7
Task 3: Neurobiological Tests of LEMP & Pb on Memory Mechanisms.....	15
Summary and Conclusions.....	20
References.....	21

INTRODUCTION

This report summarizes the work performed between September 29, 2010 and March 29, 2012 for the Air Force Office of Scientific Research and the DARPA Defense Sciences Office to assess the neurotoxic effects of low-level electromagnetic pulse (LEMP) energy, both alone and in combination with lead (Pb), on the physiological mechanisms of memory. The contributors to this effort were Dr. Douglas S. F. Ling (Principal Investigator; Associate Professor, SUNY Downstate Medical Center), Dr. Lie Yang (Research Assistant Professor, SUNY Downstate), Dr. Ronald G. Riechers (President, Spectral Energetics) and Edward Moshang, MSEE (President, EMTech Consultants, Inc.).

Traumatic brain injury (TBI) resulting from explosive blast has become a signature wound of combat operations due, in large part, to the wide use of improvised explosive devices (IEDs) by insurgent forces. Recent studies have identified a spectrum of neuropathological symptoms in U.S. warfighters who have survived IEDs attacks, including cognitive deficits, short-term memory loss, mood changes, and sleep disorders [1, 2]. In most cases, these symptoms arise in the absence of injury to other blast-susceptible organs (e.g., lungs, GI tract) and thus cannot be attributed solely to such factors as blast overpressures or thermal energies. This position is supported by recent animal-based studies which show that the neuronal damage caused by actual explosions cannot be attributed exclusively to air overpressure [3, 18]. Thus, other physical forces and pathogenic factors associated with IEDs must be considered. Two possibilities are electromagnetic (EM) energy and environmental neurotoxins, particularly lead (Pb), perhaps acting in combination.

An IED blast is a chemical reaction that generates a considerable release of energy in multiple forms, including EM. Foundational work by Keyes [4] showed that conventional explosives produce high amplitude EM pulses (up to 18,000 volts), and this has been confirmed by more recent studies examining the EM fields generated by blast detonations [12]. Metal-cased IEDs, such as adapted mortar or artillery shells, would have an especially high potential for LEMP, as the metallic casing provides a highly conductive medium for EM energy generation [4, 12]. Such energy could have neurotoxic effects. Studies of the biological effects of EM radiation, such as from microwave and radiofrequency devices, have shown that exposure to low-level EM fields can cause disruptions in neurophysiologic [13, 14] and biochemical processes [15, 16], as well as neurological and cognitive function in humans [5]. Recent studies of explosive blast neurotrauma have suggested that blast-generated EM may be an etiological factor of blast TBI [6, 17, 19]. However, there have been no studies that have examined this issue directly, and consequently, it remains unknown whether blast-generated EM contributes to the neurological damage due to explosive blasts.

Other neuropathogenic risk factors related to explosive blasts may include exposure to airborne environmental toxins, such as lead (Pb), which may derive from primers and other components commonly used in explosive ordnance. For example, the Breacher Injury Study [20], which examined the effects of repeated blast exposures to Marines training in the use of explosives, showed breacher students were not at risk for TBI. However, data from this study also showed that some students exhibited slightly elevated (though sub-toxic) levels of serum Pb [L.A Young, personal communication]. The neuropathological effects of Pb are well-established and remain a significant public health concern, as serum Pb levels as low as 10 $\mu\text{g}/100\text{ mL}$ may cause significant cognitive impairments in humans [7]. Animal studies support these concerns, having shown that environmentally relevant elevations in serum Pb lead to marked neuropathophysiology [8-10] that include depression of synaptic transmission and disruptions in long-term potentiation (LTP), the persistent form of synaptic plasticity thought to underlie learning and memory. On the battlefield, the harsh environmental conditions combined with the use of metal-

cased IEDs by insurgents may present a higher exposure risk to airborne toxins and blast-generated LEMP. Furthermore, even sub-toxic elevations in serum Pb could increase susceptibility to neurological damage by LEMP and other blast-generated forces. The aim of this pilot study was to determine whether exposure to LEMP energy, either alone or in combination with low-levels of Pb, can impair the physiological mechanisms of memory. If so, this would represent a new, previously unrecognized, health risk for U.S. warfighters serving in combat that would need to be addressed and properly mitigated.

To address this issue, we examined the effects of LEMP and Pb, both alone and in combination, on the induction of LTP of excitatory synaptic transmission in the hippocampus.

SPECIFIC AIMS AND EXPERIMENTAL APPROACH

The main objective of this study was to determine whether exposure to LEMP, alone or in combination with Pb, disrupts the physiological mechanisms of memory and may thus represent a potential neuropathogenic factor of blast TBI. To this end, electrophysiology experiments were conducted in rat brain slice preparations maintained *in vitro* to assess LTP of excitatory synaptic events. In order to expose brain slices to levels of LEMP representative of the EM energy released by conventional explosives, our experimental approach involved three main tasks. First, a computational model of blast-generated EM was developed by EMTech Consultants (Baltimore, MD) to calculate the anticipated amount of LEMP energy released by selected IED representations. The model is based on a “thin wall” stacked dielectric model of the head. Second, a custom-built EM pulse generator unit was designed and assembled by Spectral Energetics (Beavercreek, OH) to replicate blast-type EM pulses. Finally, using the EM pulse unit to generate LEMP levels computed by the mathematical model, rat brain slices were exposed to combinations of LEMP and Pb *in vitro* and then evaluated for changes in LTP using standard electrophysiology recording techniques. This approach allowed for direct assessment of LEMP-induced effects without obfuscation by other blast factors, such as air overpressure and thermal energy. The main tasks of this effort were thus organized according to the the following three aims:

1. Development of a mathematical model of blast-generated LEMP.
2. Design and testing of a laboratory EM pulse generation system.
3. Neurobiological Assessments of LEMP and Pb effects on memory mechanisms (LTP) in rat brain slices *in vitro*.

CHANGES TO ORIGINALLY PROPOSED AIMS

As originally proposed, this study was to have included examinations of LTP maintenance, i.e., how long synaptic potentiation is maintained after induction. However, the final design of the EM pulse generation system (see Figs. 2 and 11) rendered these experiments problematic, as the unit was too large to fit in the lab’s electrophysiology recording setup. Assessments of LTP maintenance require continuous monitoring of potentiated synaptic events, and for this study, such recordings would need to be acquired from the same, precise brain slice locations before *and* after LEMP exposure. Unfortunately, the configuration of the EM pulse setup required that slices be temporarily transferred from the recording chamber to the EM pulse setup for LEMP exposures, precluding continuous monitoring of the same population events pre- and post-LEMP.

In addition, we originally proposed to examine LTP in neocortex, primarily as a supplement to our planned studies of hippocampal LTP in the event hippocampal function was not affected by LEMP. However, we were unable to reliably induce LTP in our neocortical slice preparations. As shown by past studies [21], the induction of LTP in neocortex *in vitro* requires disinhibition of cortical circuits, e.g., by exposing slices to GABA_A receptor blocker such as bicuculline methiodide (BMI) or picrotoxin (PTX). However, this maneuver often results in epileptiform activity (Fig. 1) that interferes with LTP. Consequently, due to the time constraints of this pilot study, we decided to focus on hippocampus, since it is a key player in the mediation of learning and memory. Examinations of neocortex, which will be considered for future studies, may require the use of techniques to prevent epileptogenesis, such as surgical removal of the deep cortical layers or focal application of PTX (or BMI) to achieve localized disinhibition.



Figure 1. Spontaneous epileptiform burst discharges (*) in neocortical slices exposed to PTX.

EXPERIMENTAL METHODS AND RESULTS

Task 1: Mathematical Model of Explosive Blast-generated LEMP

Task 1 entailed the development of the computational model used to determine the LEMP values for brain slice experiments. All of the efforts under this task were led by EMTech Consultants (Baltimore, MD) under the direction of Edward Moshang, MSEE. The model used to determine the received EM power as it relates to various explosive IED materials was based on the complete chemical reaction of the specific explosive material under consideration. This model is capable of varying the amount of explosive to determine the EM power received at various distances from the detonation source and to relate EM pulse characteristics to the blast materials.

Task 1.1: Methods

For the present study, specific types of explosives were selected for consideration, and various RF test parameters were determined for laboratory measurement. Under consideration for this setup were explosives common to the military, e.g., RDX or TNT. The RF parameters of interest are pulse widths ranging from 0.5 – 10.0 ms and low, medium, and high frequencies of 650 MHz, 800 MHz and 1000 MHz. These frequencies were chosen because they correspond to the peaks in the spectral energy plots measured from actual detonations of conventional explosives [19]. The values computed with this model were used to guide LEMP exposures of rat brain slices in the experiments detailed under Task 3.

The model used to calculate LEMP intensity was derived from the radar design equation [22 – 24], where the received energy (E_r) is a function of the source energy (E_s) and inversely proportional to the squared distance (R) from the source: $E_r = K E_s / R^2$ (K is a constant). In terms of received power:

$$P_r = (P_t G_t)(G_p \lambda^2 \sigma) / [(4\pi)^2 R^2],$$

where P_t is the power transmitted, G_t the gain of the transmitting antenna, G_p the receiving gain, λ the wavelength of interest, and σ the aperture size through which the signal must pass to the brain. For these tests, both gains are set to unity. The value for σ is derived from the surface area of the human brain (2500 cm^2) multiplied by the attenuation factors of the stacked dielectrics, which in this case comprise the scalp, skull, and meninges [25, 26]. The key feature of this approach is that it calculates EM power at the receiver (i.e., brain), and thus allows for appropriate scaling of LEMP levels for animal testing. For example, an IED using 10 kg (22 lb) TNT has a total energy of 21.7 MJ. Most of this energy is dissipated in mechanical forms that include air overpressure, ultrasonic waves, and thermal heat. However, some is in the form of EM. The amount of energy received by the brain was selected to be compatible with published data from EM measurements of actual detonations of chemical explosives. The EM power density levels vary depending on the explosives used and are a function of frequency and pulse duration. As an example, for a pulse of 1.0 ms and 1 GHz frequency, the LEMP power received at the brain from a distance of 10 m from the source would be 2.52 KW. This would scale to 6.03 W for a rat, given a cortical surface area of 6 cm^2 . Extending this scaling further to rat brain slices of 12 mm^2 , the LEMP power would be 121 mW. In this manner, the neurotoxic effects of LEMP can be correlated for specific IED configurations. To identify and characterize the threshold levels of LEMP that are most damaging, our plan was to test scaled powers that were equivalent to levels up to 2.52 KW, frequencies from 500 – 1000 MHz, and pulse durations of 1 – 10 ms for effects on LTP.

Task 1.2: Results

The laboratory EM pulse setup developed by Spectral Energetics (under Task 2) utilized an EM pulse generator source and signal power amplifier. In this setup, the EM transmission antenna was positioned 14.5" (i.e., 0.37 m) from the targeted brain slices. Consequently, for laboratory use, the transmit power (i.e., power transmitted by the lab setup antenna) was the EM power computed at 9.63 m from the theoretical IED source, so that the power received by the slices was equivalent to the EM pulse from an IED detonation of 10 kg TNT at a range of 10 m. The following table (Table 1) shows the lab EM powers transmitted by the EM pulse generator antenna and the EM powers received by brain slices for specific EM frequencies and pulse duration of 1.0 ms. This table of values was used to set the amount of LEMP to be transmitted by the EM pulse system to illuminate the targeted rat brain slices.

Table 1. Laboratory EM Power Values

Frequency	Transmit Power (mW) (i.e., by lab setup antenna)	Received Power (mW) (i.e. EM received by slices)
500MHz	54.9	50.9
650 Mhz	37.9	29.7
800 Mhz	208	193
1000 MHz	130	121

Task 1.3: Difficulties Encountered

There were no problems encountered with this task. The model and computations of LEMP values were completed on schedule and were delivered to SUNY Downstate for the planned brain slice studies.

Task 2: Design and Testing of Laboratory EM Pulse Generation System

All of the efforts under this task were led by Spectral Energetics (Beavercreek, OH) under the direction of Dr. Ronald Riechers. This task included the design, assembly, testing, calibration, and delivery of the laboratory EM pulse generator unit that was used for the neurobiological experiments under Task 3.

Task 2.1: Methods & Results

Task 2.1.a: EM Pulse Generator Equipment Arrangement

A schematic of the EM pulse equipment is shown below (Fig. 2). The mounting surface was an air isolation table with Eccosorb flat panel absorber covering the metal surface, and a wooden mounting frame to support the ADM antenna.

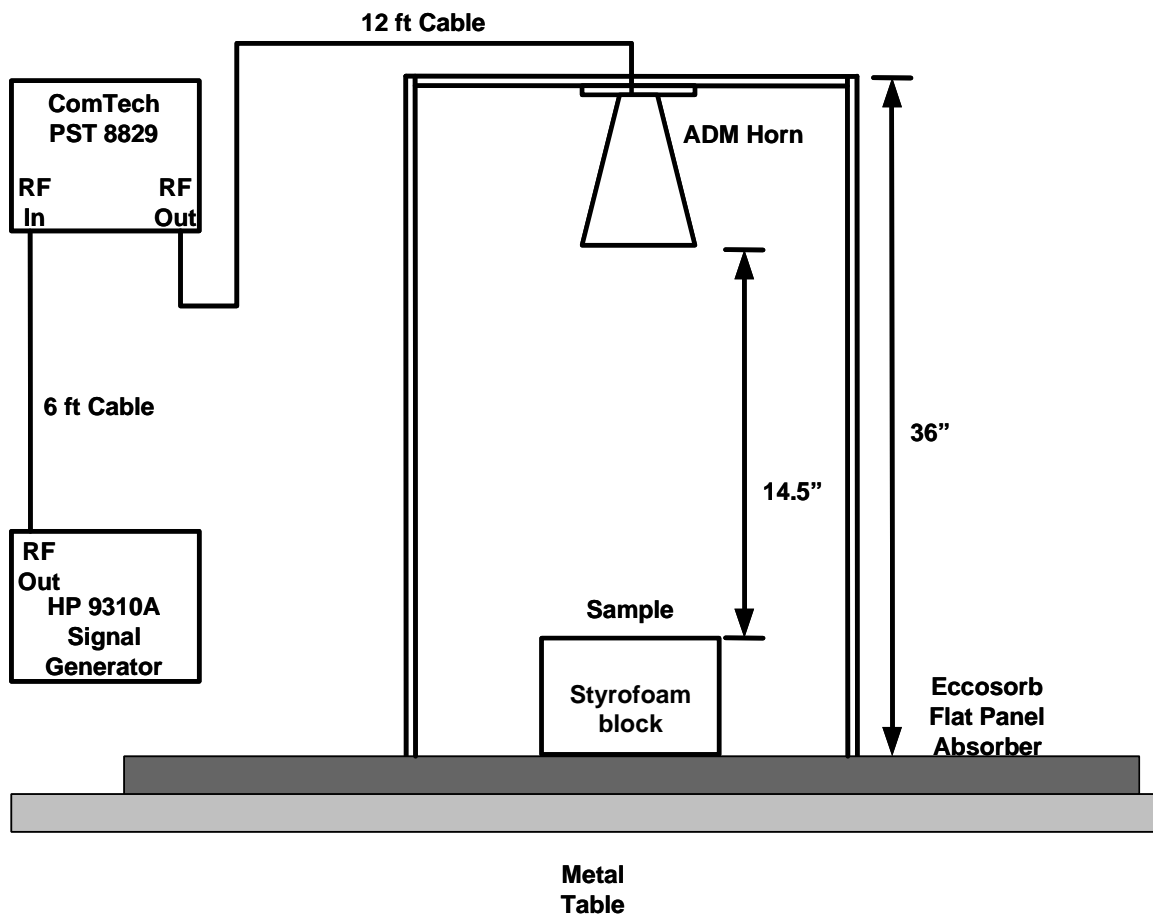


Figure 2. EM pulse set-up for exposure of brain slices to LEMP.

Task 2.1.b: Power Estimates

The maximum power density at the sample (i.e., rat brain slices) was calculated using the radar equation previously discussed under Task 1 with a continuous wave (CW) source. Once again, the power density at a range R is calculated as:

$$P_R = \frac{P_T G_{\text{amplifier}} G_{\text{antenna}} L_{\text{total}}}{4\pi R^2}$$

The transmitter power was assumed to be in the range –15 dBm to +15 dBm, the amplifier gain is +47 dB, the antenna gain is less than +3 dB, and below 1 GHz, little or no gain was expected, so the total loss was set to be 3 dB.

One issue that was raised was whether this EM setup is operating in the far field of the antenna for this short range (14.5”). The range to the far field was based on the planarity of the wave. It was assumed that the waves originated at the phase center of the ADM horn assumed to be a point source and were spherical waves. At a sufficiently large range from the source, the surface approximates a plane. The quality of the approximation depends upon the size of the illuminated target. A commonly used expression for the range is:

$$R_{FF} = \frac{2d^2}{\lambda}$$

Assuming a target size of 6 inches (i.e., large Petri dish), the range of frequencies calculated for this EM pulse generation setup are presented below.

Table 2. Range to Far Field for 6 inch Target

Frequency (MHz)	Wavelength (cm)	Range (cm)
800	37.5	12
1000	30	15
1500	20	22.5
2000	15	30

These results showed that the EM setup operates in the far field for all frequencies for the 14.5” (36.5 cm) separation distance between the antenna and the sample (i.e., targeted brain slices). Once in the far field, the antenna has formed the pattern and the system is operating in the 3 dB beamwidth for all cases. Estimates of power density were calculated as previously noted, and the table below presents a set of values based on the parameter values previously stated.

Table 3. Power Density at Petri Dish vs. Source Power

Source Power (dBm)	Power Density at Dish (W/sq meter)
-15	0.17
-5	1.7
0	5.4
+5	26
+15	270

Task 2.1.c: Component Testing

The main components of the EM pulse generation system included a signal source, a power amplifier, and antenna. The signal source selected was the Agilent N9310A to provide pulse modulated signals. The power amplifier was a ComTech PST ARD 8829-50, which was used to boost the EM signals generated by the N9310A to levels that match those calculated by the model developed under Task 1. The antenna was a Vivaldi 6 designed by Spectral Energetics to match the characteristics of the signal source and power amplifier.

Tests were performed to determine the output of the Vivaldi 6 antenna as the illuminating source for the EM pulse system to be delivered to SUNY Downstate (Fig. 3). For all tests, the Vivaldi 6 was mounted in a wood-frame mounting arrangement that would be delivered to SUNY (Fig. 3, right panel). For these tests, an Anritsu S331D SiteMaster operating in CW mode was used as the signal source. The S331D output signal was attenuated by 20 dB using fixed attenuators (Mini-Circuits, Inc), and then input to the Comtech PST power amplifier. The amplified output was sent to the Vivaldi 6 and radiated to an ADM DRH-118/A horn antenna. The output of the S331D was +4 dBm, which was reduced to -16 dBm and then input to the Comtech power amplifier. To measure all source and received power levels, the output of the ADM DRH-118/A horn antenna was attenuated and sent to an Anritsu MA24126A USB power sensor.

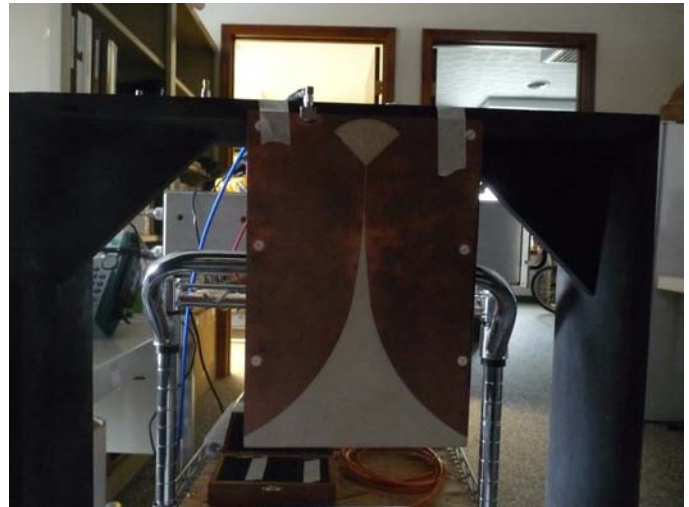
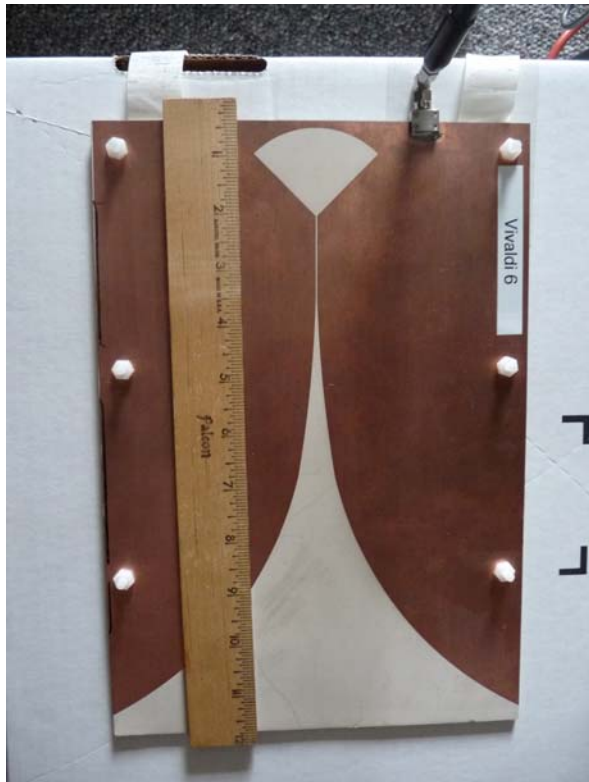


Figure 3. The Vivaldi 6 antenna (*left*) was suspended from a wood-frame arch (*right*) to illuminate the target below.

As shown in Figure 4, the output of the S331D was a function of frequency. There are additional traces that correspond to attenuated outputs that were fed to the power amplifier.

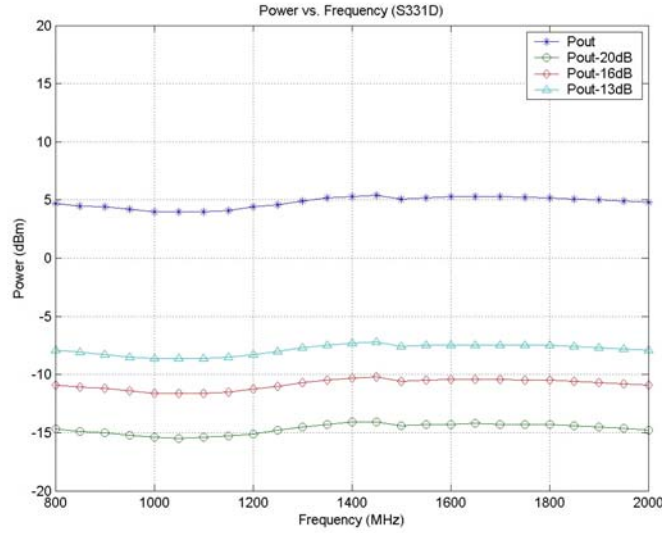


Figure 4. The output power of the S331D.

The gain (S_{21}) of the Comtech power amplifier was then measured. As shown in Figure 5, the gain of the amplifier was nearly constant over the operating band of the device, allowing the experimental values received to be in the range of 1 – 10 W with proper attenuation.

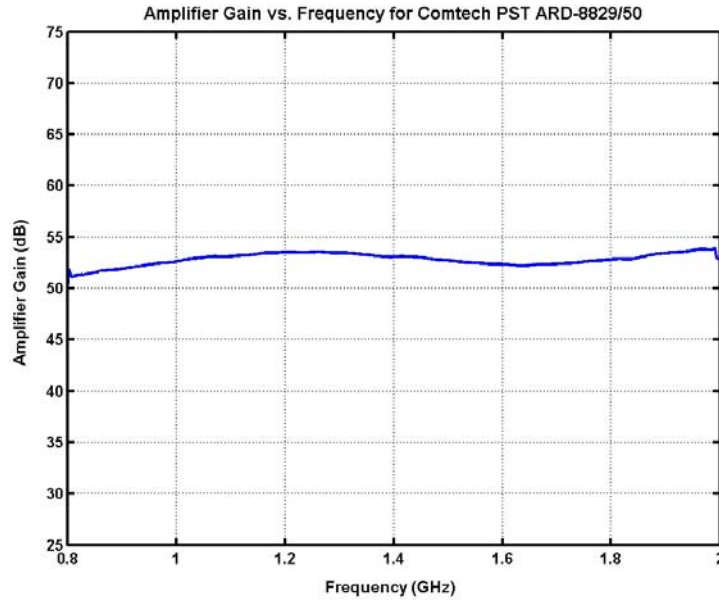


Figure 5. The gain of the Comtech power amplifier was nominally 53 dB over its operating band.

The received power was measured using the setup previously described, and the results for the three values of input attenuation are shown in Figure 6.

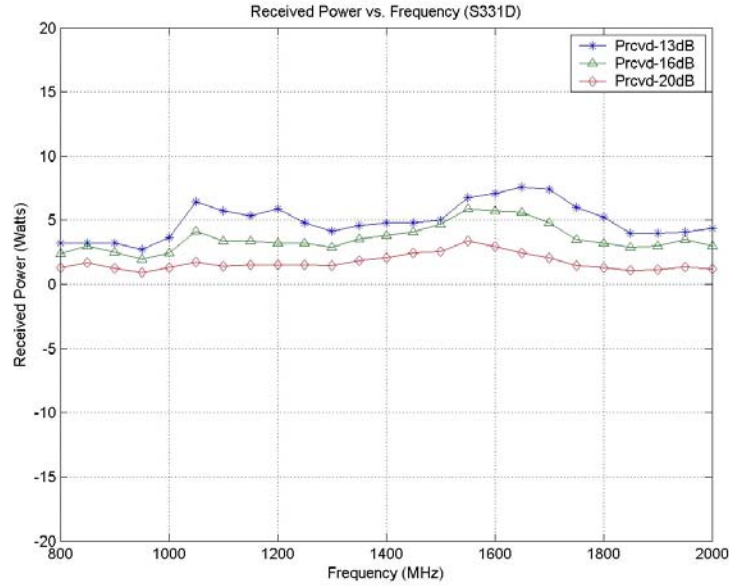


Figure 6. The received power from the amplified S331D was controlled by input attenuators.

The results of these tests indicated that the proposed system was more than adequate to provide the necessary power to the brain slice preparations to simulate the EM pulse energy generated by explosive blasts.

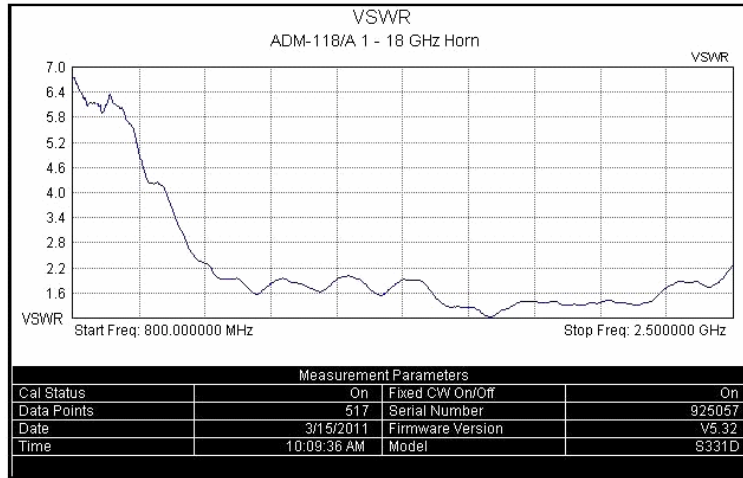
Task 2.1.d: Antenna Voltage Standing Wave Ratio (VSWR)

Tests were also conducted to measure the voltage standing wave ratio (VSWR) of each candidate antenna. For a signal source to deliver power to an antenna, the impedance of the source and transmission line must be well-matched to the antenna's impedance. The VSWR numerically describes how well the antenna's impedance is matched to impedance of the signal source and/or transmission line. The VSWRs of the candidate antennas were measured using a VNA, and the results are presented in the following figures (Figs. 7 – 10). For optimal performance, the VSWR should be below 3:1 over the operating band of the antenna, which allows for easy identification of the operating band of each unit. The ADM horn antenna was originally proposed for use in this study, but the results of these tests revealed it to have a poor performance at frequencies below 1 GHz. Consequently, the SE-designed Vivaldi antennas were substituted and used instead.

Antenna SWR

Spectral Energetics

Prepared for:
Location:
Date:



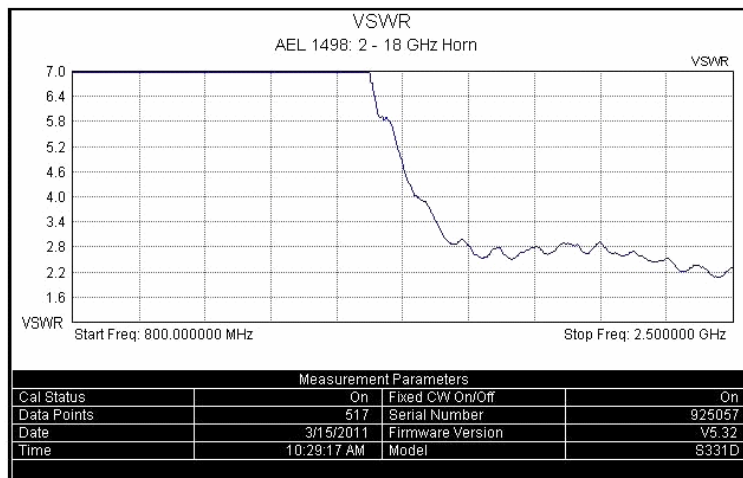
Measurement Summary			
Cal Status	On	Fixed CW On/Off	On
Data Points	517	Serial Number	925057
Date	3/15/2011	Firmware Version	V5.32
Time	10:09:36 AM	Model	S331D

Figure 7. VSWR of the ADM horn antenna

Antenna SWR

Spectral Energetics

Prepared for:
Location:
Date:



Measurement Summary			
Cal Status	On	Fixed CW On/Off	On
Data Points	517	Serial Number	925057
Date	3/15/2011	Firmware Version	V5.32
Time	10:29:17 AM	Model	S331D

Figure 8. VSWR of the AEL horn antenna.

Antenna SWR

Spectral Energetics

Prepared for:
Location:
Date:

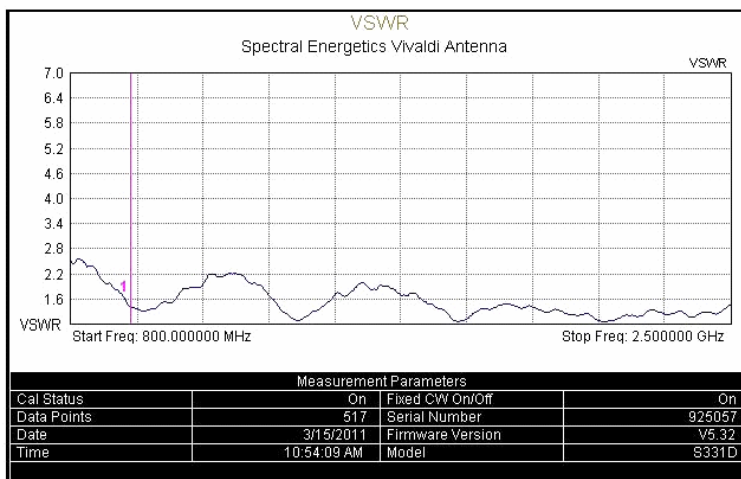
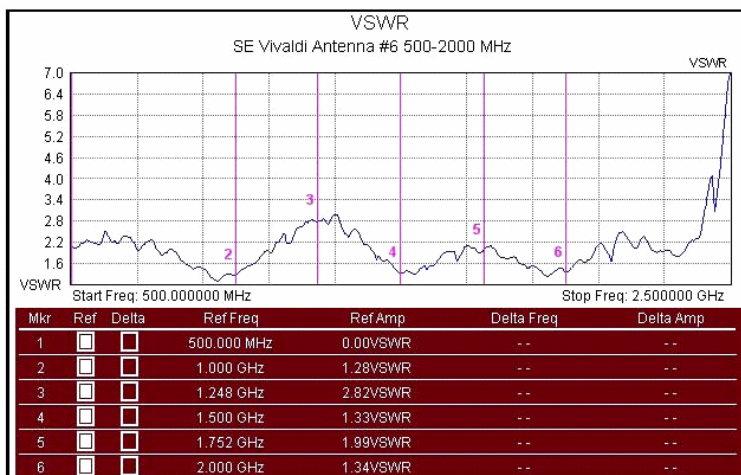


Figure 9. VSWR for the SE Vivaldi antenna

Antenna SWR

Spectral Energetics

Prepared for:
Location:
Date:



Measurement Summary			
Cal Status	On	Fixed CW On/Off	On
Data Points	517	Serial Number	925057
Date	3/15/2011	Firmware Version	V5.32
Time	2:38:09 PM	Model	S331D

Figure 10. VSWR for SE Vivaldi antenna no. 6

Task 2.2: Complete EM Pulse Generation System

Figure 11 shows the final, complete EM pulse generation system that was tested, calibrated, and delivered to SUNY Downstate Medical Center for use in brain slice experiments. The EM system was set up by SE personnel in the Laboratory of Dr. Douglas Ling in the Department of Physiology and Pharmacology.

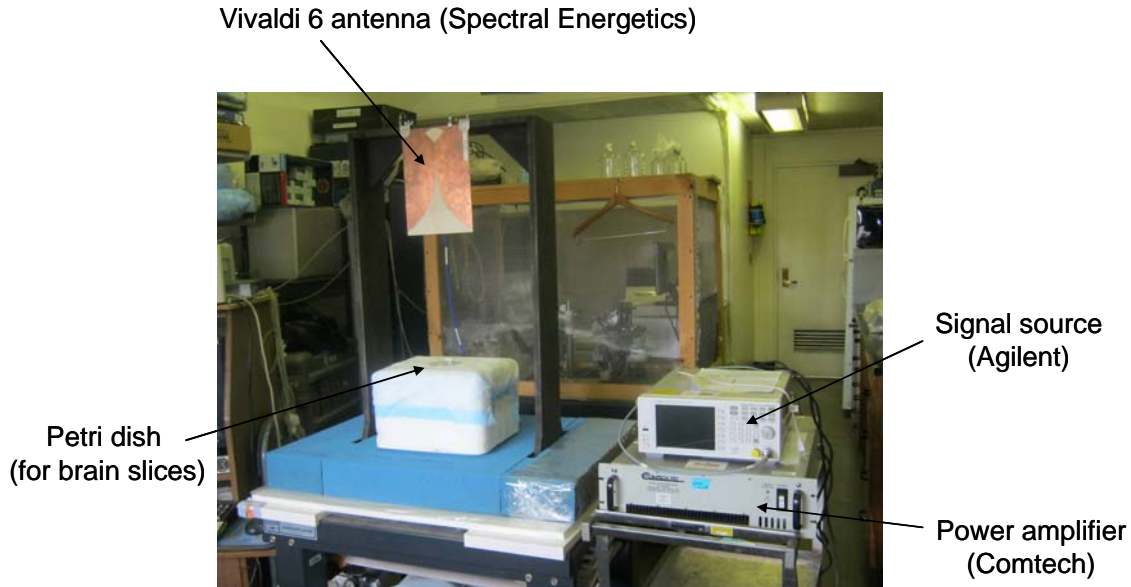


Figure 11. The EM pulse generation system at SUNY Downstate Medical Center.

Task 2.3: Difficulties Encountered

There were several problems encountered with this task. First, Spectral Energetics (SE) encountered significant delays with the order and delivery of the signal pulse generator and power amplifier from the vendor, Excalibur Engineering (Irvine, CA). In addition, once the units were received by SE, the initial choice for the signal generator unit was deemed inadequate for the study's requirements and was returned to Excalibur for exchange for a more appropriate unit, an Agilent 9310 signal generator. In all, these difficulties resulted in a 15-week delay in SE's delivery and setup of the EM pulse generator system to SUNY Downstate.

Task 3: Neurobiological Assessments of LEMP and Pb Effects on Memory Mechanisms

All of the efforts under this task were led by SUNY Downstate Medical Center (Brooklyn, NY) under the direction of Dr. Douglas Ling. This task involved the neurophysiological studies of the effects of LEMP, both alone and in combination with Pb, on the induction of LTP in rat hippocampus. Experiments used standard electrophysiological recordings of synaptic events in acutely prepared rat brain slices that were exposed *in vitro* to LEMP and Pb.

Task 3.1: Experimental Methods

Task 3.1.a: Preparation and maintenance of brain slices

All of the experimental methods used in the conduct of this study followed protocols approved by the Institutional Animal Care and Use Committee of SUNY Downstate Medical Center and the U.S. Surgeon General's Human and Animal Research Panel. All of the experiments used acute rat brain slices (450 μ m thick) that included somatosensory neocortex and dorsal hippocampus that were prepared from Sprague-Dawley rats (P21 – 30) as previously described [27]. Slices were placed in an interface recording chamber (Fine Science Tools, Foster City, CA) and maintained at $30 \pm 1^\circ\text{C}$. Slices were superfused continuously (~ 1 mL/min) with saline representing artificial cerebrospinal fluid (ACSF), which was composed of (in mM): NaCl 124, KCl 3.5, MgCl₂ 1.2, CaCl₂ 2.5, NaHCO₃ 26, D-glucose 10, continuously oxygenated with 95% O₂ and 5% CO₂ (pH 7.35-7.40). For exposure of slices to Pb, a modified ACSF was used that contained 10 – 20 μ M Pb-acetate. All slices were allowed to equilibrate in the experimental chamber for 1 – 2 h before recording. All chemicals were purchased from Sigma Chemical (St. Louis, MO).

For exposure to LEMP, slices were temporarily transferred from the experimental recording chamber to a Petri dish situated directly beneath the Vivaldi antenna on the EM pulse generation setup (see Figs. 2 and 11). Slices were exposed to a single pulse of LEMP energy and then returned to the experimental chamber, where they were allowed to re-equilibrate for ~ 30 min before application of tetanic stimulation. Control (sham-LEMP) slices were also transferred to the EM pulse setup, but did not receive any LEMP energy.

Task 3.1.b: Electrophysiological recordings

Neural population field potentials were recorded from the hippocampal CA1 region using standard extracellular techniques [28, 29]. Potentials were measured using a high impedance amplifier operating in current-clamp mode (AxoClamp 2B, Axon Instruments, Foster City, CA, USA). Microelectrodes were pulled from 1-mm thin-walled, fiber-filled capillaries. Electrodes were filled with 1 M NaCl and had tip resistances of 2-5 M Ω , and were placed in the CA1 region near the border of *stratum radiatum* and *stratum pyramidale* to record field excitatory postsynaptic potentials (EPSPs). All data signals were digitized via a Digidata 1322A and recorded directly to computer hard disk using pCLAMP 9.0 software (Axon Instruments, Foster City, CA).

Neural responses were evoked in by stimulating slices with cathodal shocks (10 – 160 μ A; 100 μ s duration) delivered via platinum/iridium electrodes (FHC, Bowdoin, ME) placed in *s. radiatum* lateral to the recording site. Stimulus intensity was systematically varied to determine threshold values for EPSPs. Slices were stimulated with single test pulses every 30 s for at least 30 min to establish baseline EPSPs. To induce LTP, theta-burst stimulation (TBS) was used that consisted of 3 trains applied at 30 s intervals, each train consisting of 3 bursts (delivered at 200 ms inter-burst intervals) of 4 stimuli at 100 Hz [21]. Test stimuli were applied up to 60 min after TBS to monitor changes in EPSP slope, which was measured from the time of EPSP onset to 0.5 ms after onset [30]. LTP was evaluated by the averaging responses at 40 – 60

min post-TBS, and averaging across experiments. Data are presented as mean \pm SEM. For statistical evaluation of data, Student's t and Mann-Whitney U-tests were performed.

Task 3.2: Results

The results of the rat brain slice recording experiments revealed two major findings. First, in the absence of Pb, exposure of slices to a single, brief pulse (1 ms) of LEMP of 800 MHz or 1000 MHz significantly inhibited the induction of hippocampal LTP. Conversely, 650 MHz LEMP was found to have no effect on LTP. However, in the presence of Pb, 650 MHz LEMP significantly inhibited LTP induction. Together, these findings indicate that specific frequencies of LEMP may be neurotoxic to the physiological mechanisms that underlie memory, and that exposure to low (sub-toxic) levels of Pb may increase susceptibility to LEMP. A detailed description of these findings is presented below.

Task 3.2.a: Hippocampal LTP: Determination of sub-toxic levels of Pb

Prior studies have shown that Pb concentrations of 5 – 20 μ M can inhibit LTP in hippocampal slices [31]. For this study, experiments were conducted to determine the “sub-toxic” levels of Pb with respect to hippocampal LTP in our brain slice system (i.e., Pb concentrations that do not affect LTP induction). In control recordings, the slopes of EPSPs recorded 60 min after TBS were increased $136.1 \pm 33.6\%$ ($n = 14$) above baseline events recorded 1 min before TBS (Fig.12A). In slices exposed to 10 μ M Pb, similar levels of potentiation were observed, with EPSPs recorded 60 min after TBS increased to $133.2 \pm 22.8\%$ ($n = 8$) above baseline, matching the LTP obtained in control slices. However exposure to 20 μ M Pb significantly inhibited LTP, with EPSPs recorded 60 min after TBS increased by only $10.1 \pm 5.9\%$ ($n = 10$) above baseline. These results indicated that 10 μ M Pb represents a sub-toxic (“low”) concentration of Pb for tetanus-induced LTP in our *in vitro* slice preparation, and would thus be used in subsequent tests for combinatorial effects with LEMP.

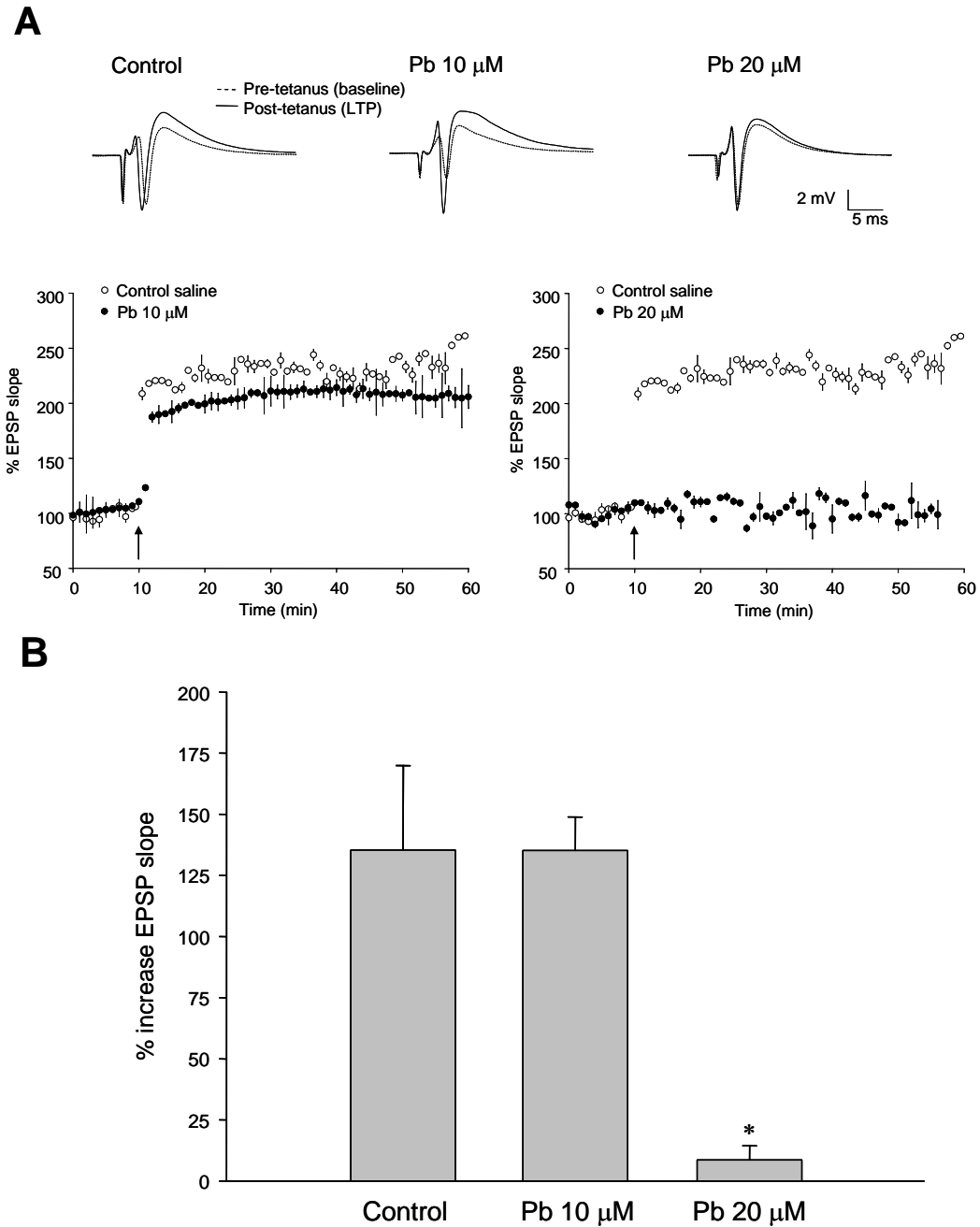


Figure 12. Effects of Pb on LTP. (a) Exposure of slices to 10 μ M Pb had no effect on TBS-induced LTP of EPSP slope, whereas 20 μ M Pb significantly attenuated induction of LTP. Arrow indicates time of tetanic stimulation. (b) Comparison of LTP in control ACSF, 10 μ M Pb, and 20 μ M Pb. * $P < 0.05$.

Task 3.2.b: Effects of LEMP on LTP induction

To test the effects of LEMP alone on LTP, slices were exposed to single, 1.0 ms duration, pulses of LEMP energy, in the absence of Pb. LEMP frequencies of 650 MHz, 800 MHz, and 1000 MHz were evaluated, and were transmitted to slices at the power levels shown in Table 1 (see Task 1). Following LEMP exposure, TBS was applied to slices and the slopes of evoked EPSPs continuously monitored to assess induction of LTP (Fig. 13). In slices exposed to 650 MHz LEMP, the slopes of EPSPs recorded 60 min after TBS were increased to $128.4 \pm 17.8\%$ ($n = 8$) above baseline, closely matching LTP levels in control slices that were unexposed

to LEMP. However, exposure to 1000 MHz LEMP significantly decreased LTP (40 min after TBS, only $42.2 \pm 21.8\%$ above baseline; $n = 9$) and 800 MHz LEMP effectively blocked LTP (40 min after TBS, $5.8 \pm 6.1\%$ above baseline; $n = 6$). These results indicate that single, brief exposures to selected frequencies of LEMP can significantly inhibit the induction of LTP. Again, it is worth noting that the frequencies tested on brain slices correspond to the peaks in the EM spectral energy plots measured from actual blast detonations [19].

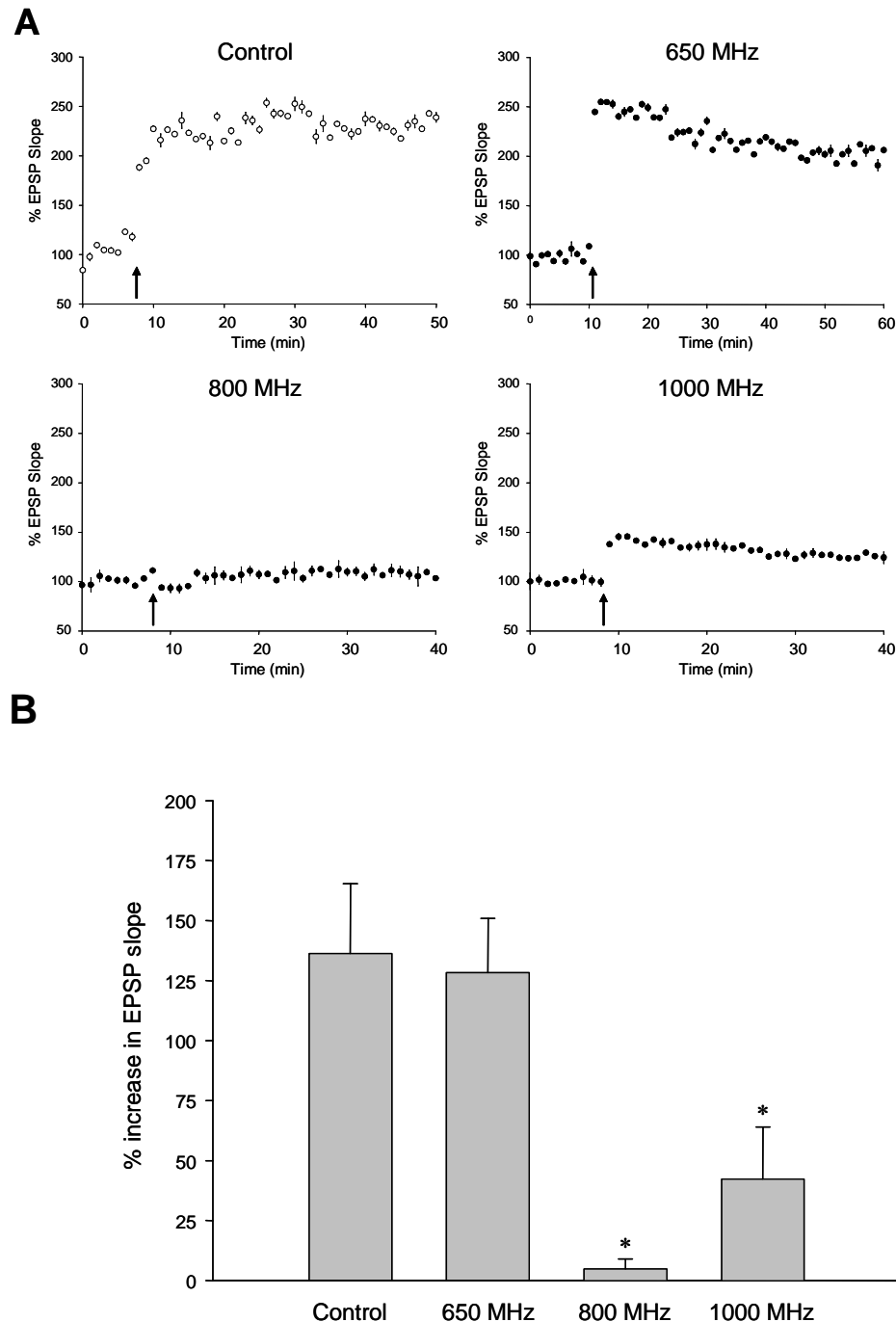


Figure 13. Effects of LEMP on LTP. (a) Exposure of hippocampal slices to 650 MHz LEMP (1.0 ms pulse) had no effect on TBS-induced LTP. However both 800 MHz and 1000 MHz LEMP significantly inhibited LTP induction. Arrows indicate time of TBS. (b) Comparison of LTP in slices exposed to 650 MHz, 800 MHz, and 1000 MHz LEMP. Control group represents sham LEMP exposure. * $P < 0.05$.

Task 3.2.c: Effects of Combined Exposure to LEMP and low Pb on LTP induction

The next experiments examined the combinatorial effects of LEMP and low-level Pb on hippocampal LTP. Slices were exposed to 650 MHz or 1000 MHz LEMP, in the presence of 10 μ M Pb. Since 800 MHz LEMP was found to effectively block LTP by itself, this frequency was excluded from this set of tests. In the presence of low Pb, 650 MHz LEMP effectively blocked LTP (Fig. 14), with EPSP slope values recorded 40 – 50 min after TBS effectively unchanged ($3.8 \pm 2.8\%$ above baseline; $n = 8$). Exposure of slices to 1000 MHz LEMP in low Pb also blocked potentiation of EPSPs (40 – 50 min after TBS, $4.6 \pm 6.4\%$ above baseline; $n = 6$), leading to a greater inhibition of LTP as compared to 1000 MHz LEMP alone. These findings suggest that exposure to low-level Pb increases the susceptibility of hippocampal circuits to the disruptive effects of LEMP.

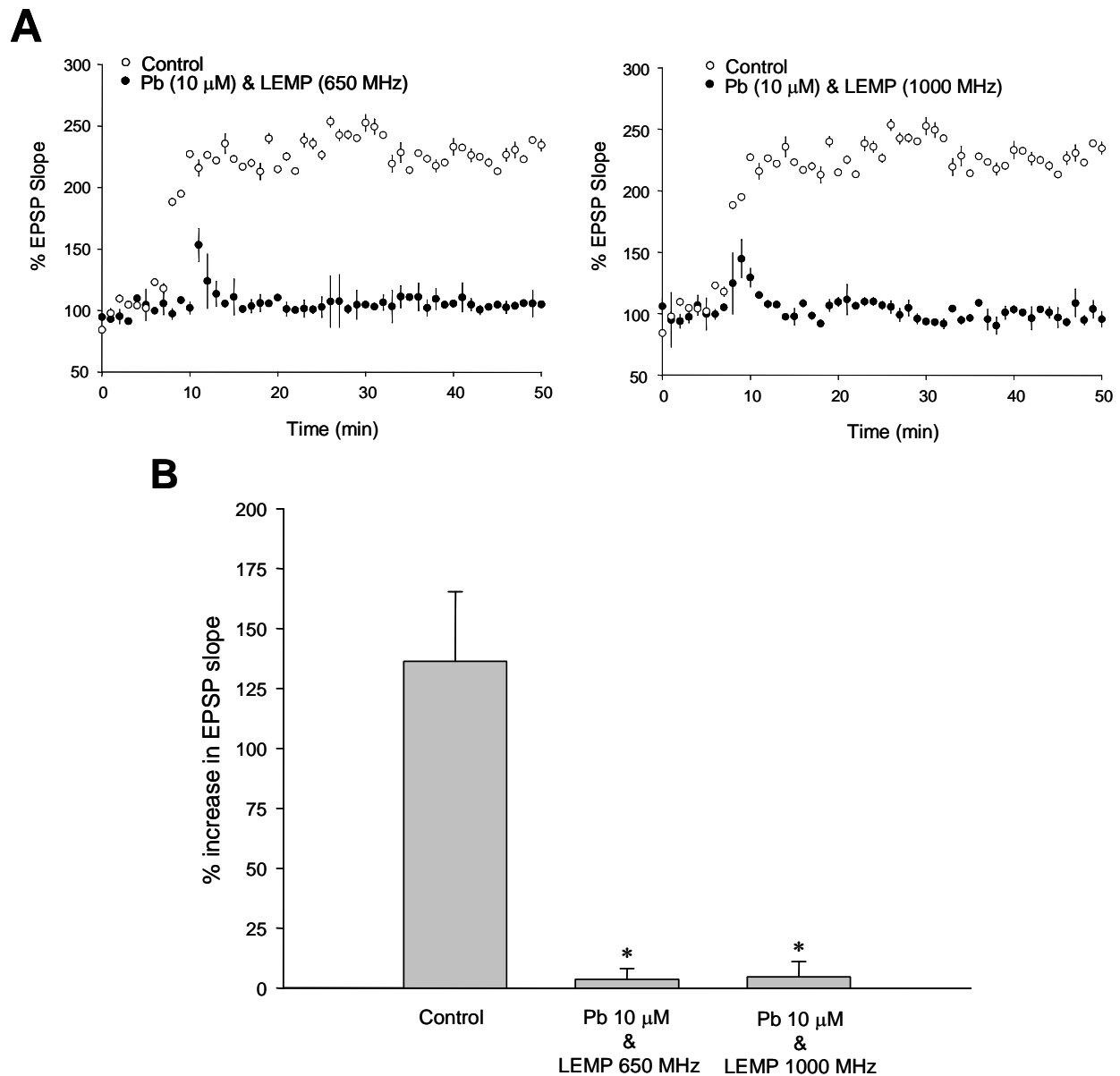


Figure 14. Effects of LEMP and Pb in combination on LTP. (a) Exposure of hippocampal slices to 650 MHz LEMP or 1000 MHz in the presence of low-level (10 μ M) blocked LTP. (b) Comparison of LTP in slices exposed to both LEMP and Pb. * $P < 0.05$.

Task 3.3: Difficulties Encountered

Two main problems were encountered with this task that necessitated modifications to our originally proposed aims, the details of which have been described above (see “Changes to Originally Proposed Aims”). First, the EM pulse unit was found to be too large to fit inside the electrophysiology rig, precluding the proposed examinations of LEMP effects on LTP maintenance. Second, we were unable to consistently induce LTP in our neocortical slice preparations and consequently, experiments focused on hippocampal LTP.

SUMMARY AND CONCLUSIONS

The results of this study showed that a single, brief exposure to LEMP can be sufficient to disrupt the induction of LTP in the hippocampus. The level of LEMP-induced inhibition appears to be frequency dependent, with 800 MHz LEMP effectively blocking LTP, while 650 MHz LEMP having no effect on synaptic potentiation. We also found that combined exposure to LEMP and low-level Pb causes a complete inhibition of LTP induction. To our knowledge, these findings represent the first evidence that LEMP energy modeled on the EM pulses generated by explosive blasts may be neurotoxic to the physiological processes of memory and learning, particularly in the presence of low Pb concentrations. Taken together, the results of this study suggest that LEMP may represent a potential neuropathogenic factor of explosive blasts, and that low levels of Pb may increase neural susceptibility to the damaging effects of LEMP.

While the data from this exploratory study support the hypothesis that blast-generated EM pulse energy could contribute to the neuropathologies caused by blast TBI, the *in vitro* brain slice preparation represents a reductionist system and as such, does not fully replicate the full spectrum of LEMP-induced effects on the intact brain or whole animal. The strength of the mathematical model developed by EMTech is its ability to scale LEMP values for any animal-based preparation, including brain slices. However, *in vivo* studies using whole animals will be needed to empirically assess the effects of blast-generated LEMP on cortical neurophysiology, anatomy, and behavior. Such information will be essential to the development of accurate, predictive models to guide the design and testing of effective countermeasures, such as improvements to body armor and helmet designs, as well as the development of effective preventive measures, diagnostic tools, and treatments for brain injuries consequent to this form of blast energy.

References

1. Okie S. Traumatic Brain Injury in the War Zone. *N Engl J Med* 352 (2005) 2043-2047.
2. Siegel G. Greater Knowledge Born from Battlefield Tragedies, *AAMC Reporter*, June 2007.
3. Laidman J. Team at University of Toledo Tackles Brain Injury Puzzle. *Toledo Blade*, Nov. 25, 2007. Comments attributed to Col. Geoffrey Ling, MD, PhD (MC, USA).
4. Keyes, RT. Electrical fields and electromagnetic radiation from chemical explosions, AFOSRTN-59-551; AD-216691; AD-216691 (1959).
5. Lai H. Neurological effects of radiofrequency electromagnetic radiation. Workshop on Possible Biological and Health of RF Electromagnetic Fields, 1998.
6. Ling G, Bandak F, Grant G, Armonda R and Ecklund J. Neurotrauma from explosive blast. In: *Explosion and Blast-Related Injuries, Effects of Explosion and Blast from Military Operations and Acts of Terrorism*, edited by N. Elsayed and J. Atkins. New York: Elsevier, 2008, p.91-104.
7. Bellinger D, Sloman J, Leviton A, Rabinowitz M, Needleman HL and Waternaux C. Low-level lead exposure and children's cognitive function in the preschool years. *Pediatrics* 87 (1991) 219-227.
8. Cory-Slechta DA, Weiss B, and Cox C. Performance and exposure indices of rats exposed to low concentrations of lead. *Toxicol. Appl. Pharmacol.* 78 (1985) 291-299.
9. Rice DC. Lead-induced changes in learning: Evidence for behavioral mechanisms from experimental animal studies, *NeuroToxicology* 14 (1993) 167-178.
10. Lasley SM, Polan-Curtain J and Armstrong DL. Chronic exposure to environmental levels of lead impairs in vivo induction of long-term potentiation in rat hippocampal dentate. *Brain Res.* 614 (1993) 347-351.
11. Lasley SM and Gilbert ME. Presynaptic glutamatergic function in dentate gyrus in vivo is diminished by chronic exposure to inorganic lead. *Brain Res.* 736 (1996) 125-134.
12. Soloviev SP, Surkov VV and Sweeney JJ. Quadrupolar electromagnetic field from detonation of high explosive charges on the ground surface, *J. Geophys. Res.* 107 (2002) no. B6, 2119, 10.1029/2001JB000296.
13. Servantie B, Servantie AM, Etienne J. Synchronization of cortical neurons by a pulsed microwave field as evidenced by spectral analysis of electrocorticograms from the white rat. *Ann NY Acad Sci.* 247 (1975) 82-86.
14. Bawin SM, Sheppard AR, Mahoney MD, Adey WR. Influences of sinusoidal electric fields on excitability in the rat hippocampal slice. *Brain Res.* 323 (1984) 227-237.

15. Adey WR, Bawin SM, Lawrence AF. Effects of weak amplitude-modulated microwave fields on calcium efflux from awake cat cerebral cortex. *Bioelectromagnetics* 3 (1982) 295–307.
16. Bawin SM, Adey WR. Sensitivity of calcium binding in cerebral tissue to weak environmental electric fields oscillating at low frequency. *Proc Natl Acad Sci USA* 73 (1976) 1999–2003.
17. Stuhmiller, JH. Blast Injury: Translating Research into Operational Medicine. In: *Quantitative Physiology: Concepts in Military Operational Medicine*, edited by WR Santee and KE Friedl. Dept of Defense, Office of The Surgeon General, US Army, Borden Institute (in press).
18. Bauman RA, Ling G, Tong L, Januszkiewicz, Agoston D, Delanerolle N, Kim Y, Ritzel D, Bell R, Ecklund J, Armonda R, Bandak F and Parks S. An introductory characterization of a combat-casualty-care relevant swine model of closed head injury resulting from exposure to explosive blast. *Journal of Neurotrauma* 26 (2009) 841-860. doi:10.1089/neu.2009.0898.
19. Young LA. Physics of open vs. enclosed space explosions. ATACCC Conference 2007.
20. Carr W, Stone JR, Young LA, Walilko Tim, Parish RV, Eonta SE, Jankosky CJ, Tsao JW and the Breacher Injury Consortium. Breacher injury study: neurocognitive testing results. 28th Annual Meeting, National Neurotrauma Symposium 2010. *Journal of Neurotrauma* 26:A-1–A-90 (May 2010). DOI: 10.1089=neu.2010.995082
21. Castro-Alamancos MA and Connors BW. Short-term synaptic enhancement and long-term potentiation in neocortex *Proc. Natl. Acad. Sci.* 93 (1996) 1335–1339.
22. Merrill G. *Principles of Missile Guidance*, Ch 4. D.Van Nostrand Co. 1955.
23. Johnson RC. *Design Notes for Microwave Antenna*, 1983.
24. Berkowitz R. *Modern Radar*, Ch 1. John Wiley & Sons, 1965.
25. Gabriel C, Gabriel S and Corthout E. The dielectric properties of biological tissues: I. Literature survey, *Phys. Med. Biol.* 41 (1996) 2231-2249.
26. Gabriel S, Lau RW and Gabriel C. The dielectric properties of biological tissues: II. Measurements in the frequency range 10 Hz to 20 GHz. *Phys. Med. Biol.* 41 (1996) 2251-2269.